Amendments to the Specification

Please replace paragraph [0095] with the following paragraph:

[0095] ~~Figure~~ FIG. 9 illustrates an electrophoresis apparatus 10 including a valving system 100 that directs the flow of fluid along a desired path through the transport capillary 24 and separation capillaries 28, 30, and 32. In this example, valves 102, 104, 106, and 108 may control the flow of buffer solution(s) around the concentrator 34; valves 106, 110, 112, and 114 may control the flow of buffer solution(s) around the concentrator 36; and valves 112, 116, 118, and 120 may control the flow of buffer solution(s) around the concentrator 38. With the valving system 100, the environment for each of the concentrator may be localized. Localizing a concentrator allows for independently controlling the microenvironment of that concentrator, such as controlling concentration of reagents, temperature, time of reactions, etc. The valving system 100 allows the loading of one or more appropriate background electrolyte solution, the introduction of the samples to be analyzed by the various modes of capillary electrophoresis, and the cleaning of the capillaries so that the capillaries may be reused.

Please replace paragraph [0099] with the following paragraph:

[0099] ~~Figure~~ FIG. 10 illustrates a perspective view of the valving system 100 for one of the analyte concentrators. Each concentrator may be surrounded by frits or porous end plates 35 provided along the path of the transport capillary 24 and the respective separation capillary to retain the matrix-like assembly 37 within the concentrator. The valves on the transport capillary and the separation capillary also surround each of the concentrators to control the flow of sample solution through the transport capillary 24 and through the respective separation capillary. The valves may be motor operated that is controlled remotely by a processor based

on a predetermined set of instructions such as a software program. After the concentrators 34, 36, and 38 have been properly conditioned, the valves along the transport capillary may be opened and the valves along the separation capillaries 28, 30, and 32 may be closed to allow the concentrated sample solution from the concentrator 17 to pass through the concentrators 34, 36, and 38. This allows each of the matrix-like assembly in the concentrators 34, 36, and 38 to bind to the desired analyte from the concentrated sample solution. The remaining concentrated sample solution may be released to the waste container 27 on the other end of the transport capillary 24.

Please replace paragraph [0101] with the following paragraph:

[0101] ~~Figure~~ FIG. 10 illustrates a concentrator with porous end plates or frits 35, which permit fluid flow, in the transport capillary 24 and separation capillary 28 to act as semi-permeable barriers for holding matrix-like assembly 37 within the analyte concentrator. For the concentrator 34, the frits 35 may be formed along the transport capillary 24 and the separation capillary 28. The frit 35 and the matrix-like assembly 37 may be the type shown in U.S. Pat. Nos. 5,202,010 and 6,406,604, which are hereby incorporated by ~~references~~ reference. The matrix-like assembly may be provided in many forms. For instance, the collective mass of the matrix may be provided by large quantities of microstructures such as beads, platelets, chips, fibers, filaments, monolithic polymers, sol-gel, or the like. Individual substrates can be made from glass, plastic, ceramic, metallic, or any appropriate polymeric chemistry compositions, and mixtures thereof. The use of interconnected beaded and/or polymeric microstructures may not require the presence of frit structures to hold the matrix, because they form a net that it is linked by chemical bonding, and they are usually positioned in a rigid configuration. In most cases, these beaded or polymerized microstructures may sustain

low-pressures. However, in certain cases that high-pressure may be needed, their network configuration can be deteriorated or destroyed. Covalently or non-covalently affinity ligands coated or immobilized onto the surface of the beaded microstructures or monolithic polymers, sol-gel, or directly onto the inner wall of the capillary, are immobilized analyte-specific antibodies or other affinity chemistries which are suitable for characterizing and separating particular analytes of interest. Representative antibodies include those which act against peptide hormones such as insulin, human growth hormone and a variety of antibodies directed against any substance of small molecular weight (classified as hapten) or any substance of larger molecular weight or biopolymer (classified as antigen). These antibodies are readily available from commercial vendors such as Sigma-Aldrich Co., St. Louis, Missouri, and Peninsula Laboratories, Belmont, California, and many other companies worldwide. Alternatively, one skilled in the art may manufacture a desire monoclonal and/or polyclonal antibody by conventional methods or protocols described in the literature. Not all haptens are capable of elicit an antigenic response by itself, usually they need to be bound to an antigenic protein carrier to generate an antibody.

Please replace paragraphs [0106] through [0109] with the following paragraphs:

[0106] ~~Figure~~ FIG. 11A illustrates a cross-sectional view of the ~~Figure~~ FIG. 10 where the valves on the transport capillary are in the second or closed position to substantially prevent the sample solution from passing towards the concentrator. The valves on the separation capillary are in the first or open position to allow the buffer solution to pass through the concentrator. The frits 35 surrounding the concentrator substantially retain the matrix-like assembly 37 within the concentrator.

[0107] ~~Figure~~ FIG. 11B illustrates that the transport capillary 24 may be staggered from one separation capillary to another to form a concentration

area 34 that is elongated. This allows additional matrix-like assembly 37 to be incorporated into the concentration area 34 to attach a desired analyte from the sample solution. In addition, the sample solution may take more time to pass through the elongated concentration area 34, which allows the matrix-like assembly additional time to bind to the desired analyte from the sample solution. The concentration area 34 may be surrounded by frits or porous end plates 35 to retain the matrix-like assembly 37 within the concentration area 34.

[0108] ~~Figure~~ FIG. 12 illustrates the steps that may be taken to concentrate, isolate, and separate the desired analytes from the sample solution provided in the sample cup 15. A first conditioning step 101 prepares the transport and separation capillaries to a desired condition. This may be accomplished by passing conditioning buffer solution through the transport and separation capillaries. The conditioning step 101 may improve the binding properties for the immobilized affinity ligands so that once the desired analyte is attracted, it is retained by the immobilized affinity ligands for as long as the optimized conditions are maintained. The conditioning buffer solution may be provided through the transport capillary 24 and/or the separation capillaries 28, 30, and 32.

[0109] Once the capillaries have been conditioned with a conditioning buffer or solution, the sample solution in the cup 15 may be introduced through the transport capillary 24. For a large capacity concentration step 103, the valve 18 may be closed and the concentrator 17 is used to obtain the concentrated sample of desired analytes. The concentrator 17 may have more surface area for greater capacity to capture the desired analytes than the other concentrators used in the valving system 100. In general, the concentrator 17 may be used for more complex matrices where several analytes may be present in the sample. For instance, the concentrator 17 may be used when hundreds or thousands of analytes are

present in the sample. On the other hand, when isolating certain compounds present in simple matrices, there may not be a need for the concentrator 17, tube 20, and waste cup 22 (depicted in ~~Figure~~ FIG. 9). Examples of simple matrices include microdialysates, artificial matrices containing standard compounds, etc. In such instances, the samples solution may be introduced directly to transport capillary 24 from the cup 15 containing the simple matrix.

Please replace paragraph [0111] with the following paragraph:

[0111] After the sample solution has been introduced into the transport capillary 24 and passed through concentrator 17, in step 105, the concentrator 17 may be cleaned. This may be accomplished by passing copious amount of cleaning buffer to the concentrator 17 followed by conditioning buffer from another cup 15', replacing cup 15, through capillary 20 and towards waste cup 22. At this stage the bound compounds to concentrator 17 can be removed or eluted out of the concentrator 17. In the elution step 107 of ~~Figure~~ FIG. 12, analytes retained by the concentrator 17 can be eluted from the concentrator 17 in many ways. One way is to pass a small amount or plug of an appropriate elution or desorption solution through the concentrator 17 to remove the bound analytes to the transport capillary 24. The bound analytes from the concentrator 17 are passed through the transport capillary 24 so that the concentrators 34, 36, and 38 may further isolate the desired analytes in each of the concentrators 34, 36, and 38. The removal of the bound compounds can be carried out as a group (simultaneously), or one or more at the time (stepwise or sequential). For isolating the desired analytes, which are cleaner or more pure and more concentrated than the original sample solution, provided in the sample cup 15, a plurality of concentrators containing more selective affinity ligands in this matrix may be used, such as concentrators 34, 36, and 38 along the transport

capillary 24 with the purpose of individually capturing a single or a more reduced number of compounds than those bound to the concentrator 17. Accordingly, there may be two concentration steps in the invention: in the first concentration step, the concentrator 17 may be used to clean or purify the sample solution from a complex mixture; and in the second concentration step, the cleaned sample solution is passed through the concentrators 34, 36, and 38 to isolate the desired analyte(s) into each of the concentrators 34, 36, and 38 to isolate the desired analyte(s) that is different than the other.

Please replace paragraphs [0119] through [0120] with the following paragraphs:

[0119] As the analytes in the concentrators 34, 36, and 38 are released in a predetermined order, the detector 46 of FIG. 1 may be movable and aligned with the separation capillary corresponding to the concentrator that the analyte is released from. For instance, with the above example, if the analyte from the concentrator 36 is released first, then the detector 46 is first aligned with the separation capillary 30 to identify the analytes released from concentrator 36. Then, the detector 46 may be repositioned to align with the separation capillary 28 to detect the analytes released from the concentrator 34, and repositioned to detect the analytes passing through capillary 32 released from concentrator 38.

[0120] The valving system may communicate with a detection system for detecting the analytes released from the concentrators. The detecting system may operate in many ways. For instance, the detection system may include a detector for each separation capillary 28, 30, and 32. In another embodiment, the three separation capillaries may be merged into one exit capillary as shown in ~~FIGURES~~ FIGS. 7 and 8, and one detector is aligned over the exit capillary. In this case, the detection system may have one detector that is fixed such that it can align over the detection window positioned in the exit capillary 66 for detecting the analytes

passing through the exit capillary. For this operation, however, additional valves may be needed to direct the separated analytes from separation capillaries 28, 30, and 32 to the single detector. For example, when separation capillary 28 is active and analytes are separated within capillary 28, capillaries 30 and 32 may be inactivated, and the separation buffers may be blocked by the corresponding valves. The fixed detectors, 86 and 88, of ~~Figure~~ FIGS. 7[[s]] and 8 may be a laser-induced fluorescence detector or a contactless electrochemical detector or a combination of similar detection devices. Furthermore, the outlet of the exit capillary may be connected to other detector systems, such as a mass spectrometer, including sample deposition onto a matrix assisted laser desorption/ionization (MALDI) plate, or a conductivity detector.

Please replace paragraphs [0123] through [0125] with the following paragraphs:

[0123] ~~Figure~~ FIG. 14 illustrates that the transport channel 24A and separation channels 28A, 30A and 32A, for the electrophoresis apparatus 10 may be formed with uniform and concave shapes that are engraved, etched or otherwise formed into a glass or plastic microchip using known lithography or other manufacturing techniques. Analyte concentrators 34A, 36A and 38A are disposed at the respective intersections of transport channel 24A and separation channels 28A, 30A and 32A with the valving system 100 to control the flow of fluid and microenvironment to each of the concentrators 24A, 36, and 38 as previously described. Near the detector 66, valves may be provided to control of fluid to the output capillary 66 from the plurality of separation capillaries. ~~Figure~~ FIG. 15 illustrates that each concentrator formed by intersection of transport and separation channels may be surrounded by valves to control the flow of liquid through the transport channel 24A and the corresponding separation channel.

[0124] ~~Figure~~ FIG. 16 illustrates a perspective view of an electrophoresis apparatus 10 having a transport channel 24A and a

plurality of separation channels 28A, 30A, 32A, and etc. Near the outlet side of the separation channels, a detector 86 may be provided that aligns with one of the detection windows of the separation channels to detect the analyte passing through the respective separation channels sequentially. To simultaneously detect the analytes passing through all of the separation channels, a detector may be provided for each separation channel to speed up the process.

[0125] ~~Figure~~ FIG. 17 illustrates that the new separation buffer solution may be added by auxiliary capillaries 122, 124, and 126 after or downstream from the concentrators in order to preserve the integrity of the antibody or any other immobilized affinity ligands. In certain applications the analytes under study may require for optimal separation from a separation buffer solution that may adversely affect the activity of the intact antibody, antibody fragment, lectin, enzyme, or any affinity ligands affected by certain compounds present in the separation buffer. Put differently, with certain separation buffer solutions may adversely affect the binding property of the immobilized affinity ligands in the concentrators so that the affinity ligands may not be used again. Also, the analytes may not be retained by the immobilized affinity ligands. With the auxiliary capillaries 122, 124, and 126, the separation buffer solution may be introduced into the separation capillaries using the cups 128, 130, and 132. This allows the separation buffer solution to flow towards the detecting zone so that there is minimal, if any, interaction between the separation buffer solution and the antibody in the concentrator. For example, the separation of an analyte may require the presence of organic solvents or other additives in the separation buffer solution such as urea, certain detergents, etc. If such separation buffer solution passes through the concentrator so that the separation solution interacts with the antibody in the concentrator, the separation buffer solution may disrupt the binding process between the analyte and the antibody during the conditioning

process of the capillary and/or destroy the quality of the antibody in an irreversible manner. Such adverse effect on the antibody may destroy the integrity of the binding capacity of the antibody so that it may not bind to the analyte and/or may not be used again. To substantially prevent such adverse effect on the antibody, the antibody in the concentrator is isolated from such separation buffer solution to protect the immobilized antibody, or antibody fragments or other affinity element, such as a lectin or an enzyme.

Please replace paragraph [0127] with the following paragraph:

[0127] ~~Figure~~ FIG. 17 illustrates cups 128, 130, and 132 located on the second stage of the separation capillaries 28, 30, and 32. The cups 128, 130, and 132 may be coupled to the corresponding separation capillaries through auxiliary capillaries 122, 124, and 126, respectively. The cups 128, 130, and 132 may hold separation buffer solutions that are feed into the separation capillaries 28, 30, and 32 downstream from the concentrators 34, 36, and 38, respectively. The auxiliary capillaries 122, 124, and 126 used to couple the cups 128, 130, and 132 to the separation capillaries 28, 30, and 32 may be electrolyte-provider capillaries (EPCs). The auxiliary capillaries 122, 124, and 126 may be coupled to the respective separation capillaries 28, 30, 32, downstream or after the concentrators 34, 36, and 38 so that the buffer solutions flow towards the detecting window 45. The auxiliary capillaries 122, 124, and 126 may be also coupled to the valves 108, 114, and 120 downstream from the concentrators 34, 36, and 38 to control the flow of the buffer solution into the separation capillaries 28, 30, and 32 by opening and closing the valves 108, 114, and 120. This way, the buffer solutions generally do not interact with the immobilized antibodies in the concentrators 34, 36, and 38. With the cups 128, 130, and 132 positioned downstream from the concentrators in the apparatus 10, the separation buffer may be

introduced into the apparatus 10 either before the concentrators using the cups 40, 42, and 44, or after the concentrators using the cups 128, 130, and 132, depending on the interfering of the separation buffer on the binding between the analyte(s) of interest and the immobilized affinity ligands in the concentrators 34, 36, and 38 and/or the damage that the constituents of the separation buffer can do to the immobilized affinity ligands.

Please replace paragraphs [0130] through [0135] with the following paragraphs:

[0130] ~~Figure~~ FIG. 18 illustrates another embodiment of electrophoresis apparatus 10, configured to capture and detect primarily large sized particles such as cells, organelles, and/or other bulky globule structures. The large particles may require a larger cross-sectional area for the particles to pass through without blockage or interference during separation. The configuration where the affinity ligands are immobilized on the surface of a bead, or cross-linked, or on monolithic structures may not be appropriate for the separation of globule structures. The blockage may occur in such situations and may prevent the separation of such structures from occurring. This embodiment may also be used to capture and detect small molecules and bio-molecules.

[0131] ~~Figures~~ FIGS. 18 and 19 illustrate the electrophoresis apparatus 10 having matrix-like assembly antibodies along the interior surfaces of the separation capillaries 28, 30, and 32. That is, the affinity 37 elements may be also covalently bonded directly to the inner wall of the capillary or to beads covalently bound to each other and also bound to the inner wall of the capillary. The use of covalent bonds to bind beads within a matrix is also described in U.S. ~~Patent~~ Pat. No. 5,202,010, which is referred to as beaded capillaries. The attachment of beads to the capillary through covalent bonds may produce strong bonds that can hold the beads in the predetermined location along the capillary.

[0132] ~~Figure~~ FIG. 20 illustrates the process undertaken to isolate the monovalent antibody fragment Fab'. The antibodies may be obtained by subjecting purified IgG antibody to two partial enzymatic digestions to obtain F(ab')2 fragment. The resulting F(ab')2 antibody fragment may be further reduced to produce monovalent Fab' antibody fragments. As shown in ~~Figure~~ FIG. 21, the Fab' antibody fragment attaches to the inner wall of the capillary by creating cross-links or bridge chemistries between a sulfhydryl group of the antibody fragment Fab' and an amino group of a chemical arm bound to the silanol groups of the inner surface of the fused-silica (quartz) capillary or the surface of beaded structures or polymeric microstructures having terminal silanol groups. The antibody fragments attaches to the surface of the separation capillary in an orientation that facilitates the binding of the antibody and the desired analyte. A proper orientation of the Fab' antibody fragments results in an increased surface area of the analyte-concentrator to provide greater capacity to capture the desired target analyte. A number of antibodies that have affinity to a predetermined antigen or hapten may be provided along a predetermined portion of one or more separation capillaries 28, 30, and/or 32. An antigen is a chemical compound that normally causes the body to produce an antibody when the immunological system in the body recognizes it. A hapten is a chemical compound that normally does not produce an antibody because it is too small and may not be recognized by the immunological system. To produce an antibody for a hapten, the hapten may be bound to an immunogenic carrier (e.g., albumin, hemocyanin, etc.). This may allow the immunological system to recognize the package (hapten-carrier) as foreign, causing the development of an antibody. As discussed above, the concentrator 17 may provide a number of analytes of interest to the valving system 100 through the transport capillary 24. To identify the predetermined number of analytes of interest, each separation capillary 28, 30, and 32 may be provided with an antibody that has affinity

to a particular analyte. For example, as illustrated in ~~Figure~~ FIG. 18, a first type of antibodies 140 that have affinity to a first analyte provided by the concentrator 17 may be provided within the interior wall of the separation capillary 28. Likewise, a second type of antibodies 142 and a third type of antibodies 144 that have affinity to a second analyte and third analyte may be provided within the interior walls of the separation capillaries 30 and 32, respectively.

[0133] ~~Figure~~ FIG. 18 illustrates a valving system 100 that allows the concentrated analytes from the concentrator 17 to pass through the first, second, and third antibodies 140, 142, and 144. The transport capillary 24 may be staggered from one separation capillary to another to form an elongated analyte concentrator. For instance, the transport capillary 24 is staggered at the separation capillaries 28, 30, and 32 forming elongated analyte concentrators 140, 142, and 144. To pass the concentrated analytes through the valving system 100, the valves 104, 108, 110, 114, 116, and 120 along the separation capillaries 28, 30, and 32 may be closed, and the valves 102, 106, 112, and 118 along the transport capillary 24 may be opened. Once the output valve 18 is opened, and the analytes bound to the concentrator 17 are eluted, as described in step 107 in ~~Figure~~ FIG. 12, so that the concentrated analytes of interest flow through the first, second, and third types of antibodies 140, 142, and 144. As such, the antibodies that have affinity to a particular type of analyte may bind to that analyte. For example, as the concentrated analytes pass through the first antibodies 140, the first analytes of interest from the concentrated analytes from the concentrator 17 couple to the first antibodies 140, then as the remaining concentrated analytes pass through the second and third antibodies 142 and 144, the second and third analytes of interest couple to the second and third antibodies, respectively. The remaining concentrated analytes can then be discarded to the waste container 27.

[0134] With the desired analytes bound to the antibodies 140, 142, and 144, the conditioning, separating and eluting buffer solution from the cups 40, 40', 40", 42, 42', 42", and 44, 44', 44" may be provided to the immobilized antibodies or antibody fragments, to release and separate the bound analytes from the immuno complex. This may be accomplished by closing the valves 102, 106, 112, and 118 along the transport capillary 24, and opening the valves 104, 108, 110, 114, 116, and 120 to provide the separation buffer solutions from the cups 40, 42, and 44. For the separating step, the separating buffer solution may be provided either through the cups 40, 42, and 44 or through the cups 128, 130, and 132 as discussed above in ~~Figure~~ FIG. 17. To capture cells, organelles, and/or other bulky structures, the concentrator 17 may not be needed.

[0135] ~~Figure~~ FIG. 19 also illustrates the addition of valves 152, 154, and 156 to control the flow of buffer solutions in cups 128, 130, and 132 into the respective separation capillaries 28, 30, and 32. The valves 108, 114, and 120 are opened when the capillaries 28, 30, and 32 are filled with conditioning buffer from cups 40, 42, and 44. Then the valves 152, 154, and 156 may be opened to allow the separation buffer from the cups 128, 130, 132 to enter into the respective auxiliary capillaries 28, 30, and 32. As the electric charge creates an electroosmotic flow in the direction of the detection zone, the separation buffer entering the capillaries 28, 30, and 32 downstream from the concentrators flow towards the detection zone as well. The electroosmotic flow created by the electricity moves the analytes along the separation buffer towards the detection system, allowing separation of the elements to take place.

Please replace paragraph [0138] with the following paragraph:

[0138] ~~Figure~~ FIG. 22 illustrates a separation capillary 28 having more than one type of antibodies within its interior wall between the valves 104 and 108. The separation capillary 28 may be divided into many portions,

where each portion has one type of antibodies to isolate a particular type of analyte. For example, the separation capillary 28 may have different types of antibodies 140, 150, and 160 each having affinity to different type of analyte. As such, the separation capillary may isolate a number of different types of analytes. The separation capillary 28 may be elongated to incorporate more antibodies if desired. The transport capillary 24 may be coupled to the separation capillary 28 near the valve 108 to provide the concentrated analytes from the concentrator 17. As the concentrated analytes pass through the separation capillary 28, each of the antibodies may couple to the desired analytes.

Please replace paragraphs [0140] through [0144] with the following paragraphs:

[0140] The antibody may be any type of affinity interacting chemical or biological system that attracts a particular analyte. ~~Figures~~ FIGS. 23A and 23B illustrate an enlarge view of the antibodies 140, 150, and 160 along the interior surface of the separation capillary 28. Each antibody generally has a shape that is coupled to a substrate, which in this case is the interior surface of the separation capillary 28. The Y shape antibody includes two arms and one stem that imbeds into the substrate. As such, the antibody is immobile, but the two arms have affinity for a particular analyte (one in each arm) and as that analyte passes across the antibody, the two arms bond to the analyte until the eluting buffer solution interacts with the antibody to release the analyte. For example, in ~~Figure~~ FIG. 23A, the two arms for the antibodies 140 have affinity for the circular analyte but not the square analytes or the triangular analytes. In contrast, the two branches for the antibodies 160 have affinity for the square analyte but not the circular analytes or the triangular analytes. Other antibodies in the separation capillaries 28, however, may have affinity for the triangular analytes and bond to the triangular analytes.

[0141] ~~Figure~~ FIG. 23B illustrates polymeric microstructures with Y shape antibody having affinity for a particular analyte within the concentrator area without the need for frits. Each beaded microstructure may have an antibody that has affinity for a different analyte.

[0142] ~~Figures~~ FIGS. 24A and 24B illustrate the use of an antibody like Fab' as described above. In contrast to the antibodies shown in ~~Figures~~ FIGS. 23A and 23B, these Fab' antibodies have one side of the original antibody. The antibodies are attached to the substrate by a portion of the original stem, allowing each group of antibodies to retain their specificity, attracting and bonding to only one type of analyte.

[0143] ~~Figures~~ FIGS. 25-27 illustrate a microextraction device 200 having four tubing-connecting ports: two ports 210 couple to the transport capillary 24, and two other ports 214 couple to separation capillary 28, for example. The two ports 210 for the transport capillary 24 may be larger than the two ports 214 for the separation capillary to accommodate the larger size opening in the transport capillary 24. Port 210 may be formed from fused-silica, port 214 may be formed from a plastic tube. As illustrated in ~~Figure~~ FIG. 27A, the two ports 210 and 214 intersect to form a concentration area 246. The microextraction device 200 may also have a filling port 252 that provides access to the concentration area 246. The filling port 252 may be provided at the central part of the microextraction device 200. With the filing port 252, prepared by using controlled pore glass (CPG) beads having covalently attached antibody fragments to their surfaces may be inserted into the concentration area 246. This feature allows the coated beads to be replaced as the performance of the immobilized antibody fragments degrades after repeated usage.

[0144] The ports 210 and 214 may be formed within the base 202, and the filing port 252 may be formed on the cover 208. The base 202 may have openings 230, 232, 234, and 236 that pass through the

corresponding ports 210 and 214. The openings 230, 232, 234, and 236 may be adapted to receive the elongated portion of valves 218, 220, 222, and 224 that are able to move between first and second positions. As illustrated in ~~Figure~~ FIG. 25, each valve may have a protruding portion 226 with a cutout 228 to control the flow of fluid through the respective capillary. The cutout 228 may also be a hole found through the protruding portion 226. The hole may be coated with glass. To enable normal electrosmotic flow of liquid through the cutout 228, the cutout 228 may be formed from fused silica or coated with fused silica to maintain a closed connection with the fused-silica capillaries for the CPG beads.

Please replace paragraphs [0146] through [0150] with the following paragraphs:

[0146] As illustrated in ~~Figure~~ FIG. 25, an indicating arrow 238 may be provided on the valve so that if the direction of the indicating arrow is in line with longitudinal axis of the capillary then the valve is in the first position, and if the direction of the indicating arrow is perpendicular to the longitudinal axis of the capillary then the valve is the second position. In the first position, the cut out 228 is aligned with the longitudinal direction of the port to allow the fluid to pass through the port. In the second position, however, the cut out 228 faces away from the port so that the protruding portion of the valve blocks the flow of fluid through the port. A connector 240 may be provided to couple the microextraction device 200 to the transport and separation capillaries. For instance, in ~~Figure~~ FIG. 26, the connector 240 may be used to couple the capillary 28 to the port 210 so that the fluid from the capillary 28 may be passed to the port 210.

[0147] ~~Figure~~ FIG. 26 illustrates a cut out view of the intersection area 246 formed by the intersection of the ports 210 and 214. This way, if the valves 218 and 222 are in the first position and the valves 220 and 224 are in the second position, the fluid from the capillary 28 may pass through the port 210, and then to the capillary 28 on the other end of the base 202

towards the detection device. Likewise, if the valves 220 and 224 are in the first position and the valves 218 and 220 are in the second position, the fluid from the capillary 24 may pass through the ports 214 and 216, and then to the transport capillary 24 on the other end of the base 202.

[0148] As further illustrated in ~~Figure~~ FIG. 27A, the intersection area 246 may have bulging members 248 along the corners of the channels 210, 212, 214, and 216. ~~Figure~~ FIG. 27B is an enlarged view of the intersection area 246 illustrated in ~~Figure~~ FIG. 27A. The bulging members 248 along the ports provide for a restricted area in the area 246 such that the gaps through the ports in the intersection area are smaller than the size of the beads or matrix 250, thereby preventing the beads or matrix from moving out of the intersection area 246. As such, the beads or the matrix may capture the desired analytes as the sample passes through the intersection area 246.

[0149] As illustrated in ~~Figures~~ FIGS. 28A and 28B, the port 210 that is aligned with the transport capillary may be staggered to form an elongated concentration area 246. This allows additional matrix-like assembly or beads 250 to be incorporated into the concentration area 246 to attract the desired analyte from the sample solution. In addition, the bulging members 248 may be provided near the intersection area 246 to contain the beads within the intersection area.

[0150] ~~Figure~~ FIG. 25 illustrates that the cover 208 may have a filling port 252 adapted to receive a cap 254. The filling port 252 may be provided to insert the beads 250 or matrix into the intersection area 246 to capture the desired analytes. Once the beads 250 are inserted into the intersection area, the cap 254 may be used to enclose the filling port 252. Moreover, the beads 250 may be replaced through a variety of methods. For instance, the beads in the intersection area 246 may be removed by opening the cap 254 so that the beads are exposed through the filling port

252. The beads may then be removed through a vacuum source such as a syringe. Once the old beads are removed, a new set of beads with affinity for a desired analyte may be inserted to the intersection area 246 through the filling port 254. To secure the new beads within the intersection area, the cap 254 may enclose the filling port 252.

Please replace paragraph [0156] with the following paragraph:

[0156] All chemicals were of the highest quality reagent grade. Deionized, double-distilled water was purified with a ~~Milli-Q-Plus~~ MILLI-Q® Plus Ultra-Pure water system from Millipore Corporation (Bedford, MA, USA). Nylon filters (0.20 μm) used to remove particulate matter were obtained from Gelman Sciences (Ann Arbor, MI, USA). Underivatized controlled pore glass (CPG) beads (3000 Å pore size, 200-400 mesh, irregularly shaped) were purchased from CPG Inc. (Fairfield, NJ, USA). Bare fused-silica capillary columns were obtained from Polymicro Technologies (Phoenix, AZ, USA). Sulfosuccinimidyl 4-(N-maleidomethyl) cyclohexane-1-carboxylate (SSMCC), the immunoPure F(ab')2 preparation kit, 2-mercaptoethylamine.HCl, ~~Blue Carrier®~~ BLUE CARRIER® immunogenic protein, and pepsin agarose were purchased from Pierce Biotechnology (Rockford, IL, USA). 3-Aminopropyl-triethoxysilane was obtained from Polysciences (Warrington, PA, USA). S-(+)-Ibuprofen ((S)-(+)-2-(4-isobutylphenyl)propionic acid), S-(+)-naproxen ((S)-(+)-2-(6-methoxy-2-naphthyl)propionic acid), phenylmethylsulfonyl fluoride, soybean trypsin inhibitor, iodoacetate, p-aminobenzamidine.HCL, leupeptin hydrochloride, potassium chloride, sodium phosphate (Na2PO4), and potassium phosphate (KH2PO4) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Peptide-N-glycosidase F (PNGase F) was obtained from New England Biolabs (Beverly, MA, USA). Sodium thiocyanate and sodium azide were purchased from Fisher Scientific (Pittsburgh, PA, USA). Superdex-75

resin, PD-10 desalting column, and r-Protein A ~~Sepharose™~~ SEPHAROSE® were purchased from Amersham Pharmacia Biotech (Piscataway, NJ, USA). Angiotensin II and neurotensin were obtained from Peninsula Laboratories (Belmont, CA, USA) and Sigma-Aldrich. Methanol was purchased from Allied Signal, Burdick & Jackson (Muskegon, MI, USA). ~~Sep-Pak~~ SEP-PAK® C18 cartridges were obtained from Waters Corporation (Milford, MA, USA). A concentrated (10-fold) phosphate-buffered saline solution was prepared as follows: Dissolve 80 g of NaCl, 2.0 g of KCl, 14.4 g of Na2HPO4, and 2.4 g of KH2PO4 in 800 ml deionized, double-distilled water; adjust the pH to 7.40 with HCl; adjust the volume to 1 liter with additional deionized, double-distilled water; filtered with Nylon filters (0.20 μm). The final concentration of the used phosphate-buffered saline (a 1:10 dilution from the concentrated) is approximately the following: 0.010 M sodium phosphate (dibasic) buffer, pH 7.40, containing 0.0027 M potassium chloride, 0.137 M sodium chloride, and 0.0018 M potassium phosphate (monobasic).

Please replace paragraphs [0162] through [0163] with the following paragraphs:

[0162] Polyclonal antibodies raised against commercially available ibuprofen, naproxen, angiotensin II, and neurotensin (~~Figures~~ FIGS. 29A and 29B), coupled covalently to ~~Blue Carrier~~ BLUE CARRIER® immunogenic protein, were raised in rabbits using a method similar to those described elsewhere [3,4]. The purification of the antibodies from rabbit antisera was performed by HPLC using r-Protein A affinity chromatography as previously described [4]. Conjugates for immunization were prepared by various methods with the same modifications [1,5-7]. The method described by Grafe and Hoffmann [5] was used to link ibuprofen to the ~~Blue Carrier~~ BLUE CARRIER® immunogenic protein. The method described by Shi *et al.* [6] was used to link naprofen to the

immunogenic protein. The linking of the peptides to the immunogenic protein was performed as previously reported for other carriers [1,7].

[0163] The antibodies purified from the antisera by r-Protein A ~~Sepharose~~ SEPHAROSE® affinity chromatography, were further purified by immunoadsorption on a CPG column containing immobilized haptens (ibuprofen, naproxen, angiotensin II, or neurotensin). In order to accomplish this task, the haptens ibuprofen and naproxen were covalently linked to controlled-porous glass, employing the same chemistries used to link the haptens to the ~~Blue Carrier~~ BLUE CARRIER® immunogenic protein. The peptides were linked to CPG through a procedure described elsewhere [7]. The columns were individually eluted with 3 M sodium thiocyanate in 0.01 M sodium phosphate buffer pH 7.0 [3]. The highly specific, pure antibodies were dialyzed against 0.01 M sodium phosphate buffer pH 7.0, aliquoted in small fractions, and stored at -70°C until use.

Please replace paragraphs [0165] through [0166] with the following paragraphs:

[0165] Immunoadsorbed purified antibodies were subjected to two partial enzymatic digestions to generate F(ab')2 antibody fragments. The first digestion, a deglycosylation process described by Wilson et al. [8], was performed to remove N-linked glycosyl groups attached to the Fc fragment of IgG. These investigators used PNGase F, an enzyme that removes N-linked oligosaccharides. Approximately 20 U/μL PNGase F were incubated with 1 mg/mL of purified IgG for 24 hr at 37°C (~~Figure~~ FIG. 20).

[0166] The second enzymatic digestion was carried out using pepsin to remove the Fc fragment of the IgG while maintaining the intra- and inter-disulfide bridges. This enzymatic process was readily achieved, since the Fc fragment was free of sugars. The removal of some steric hindrance from neighboring carbohydrate moieties near the hinge region facilitated

the action of pepsin. Pepsinolysis was carried out using a combination of the method described by Wilson et al. [8], and the manufacturer's instructions described in the ImmunoPure F(ab')2 preparation. The divalent F(ab')2 antibody fragments formed were then reduced to monovalent Fab' antibody fragments, by incubation with equal volumes of 200 mM mercaptoethylamine.HCl reagent for 30 min at 37°C. This step, reported by Phillips and Smith [2], replaced the F(ab')2 reduction with Cleland's reagent (as previously described) because the latter agent was found to require optimization conditions and it is or can be an unpredictable reducing agent (~~Figure~~ FIG. 16).

Please replace paragraph [0168] with the following paragraph:

[0168] Controlled-porous glass beads, previously utilized to link antibodies directed against methamphetamine [7,9], were employed to bind monovalent Fab' fragments purified from antibodies raised against the two acidic drugs and the two neuropeptides [9,10,1,7,11]. The irregularly shaped beads were incubated at 95°C for 60 min in the presence of 10% aqueous 3-aminopropyltriethoxysilane. This treatment was repeated four times. The incubation was carried out with the beads and solution inside a double side arm glass container, in a temperature-controlled water bath, with gentle agitation. The access ports of the glass container, inlet and outlet, were sealed with multi-hole plastic caps to reduce the evaporation of the silane solution. The beads were then incubated at 95°C for 60 min with 10 mM hydrochloric acid. The beads were washed with copious amounts of distilled/deionized water before preparing the maleimide-activated surface. The beads were then incubated at 30°C for 60 min with a buffer solution containing 50 mM sodium borate, pH 7.6, and 1 mg/mL SSMCC. The beads were finally washed thoroughly with 50 mM sodium borate buffer, pH 7.6, and then incubated overnight at 4°C with approximately 500 μg/mL of SH-

containing Fab' peptide in 50 mM sodium borate buffer, pH 7.6. The entire process to link SH-containing Fab' fragments to the wall of the capillary is summarized in ~~Figure~~ FIG. 21.

Please replace paragraphs [0170] through [0172] with the following paragraphs:

[0170] The analyte concentrator-microreactor device, designed in a cruciform configuration with four entrance-exit ports (~~Figure~~ FIG. 11A), was made of a transparent acrylic substrate, but other plastic materials (e.g., ~~Teflon®~~ TEFLON® fluoropolymer resins, nylon, polyimide, or PEEK® plastic) may also be used. Two chromatographic fittings were used to connect two large-bore plastic tubes (one inlet, one outlet) to the microextraction device to transport sample and washing buffers. Two nanovolume fittings (nomenclature used to describe a device or sleeve to provide a tight fitting to a fused-silica capillary) were used to connect two 100-μm i.d., 360-μm o.d. fused-silica capillaries (one short inlet, one long outlet) to the device (~~Figures~~ FIGS. 25-27). Each port contained a porous polymeric frit, or a constricted area, fabricated from fine pieces of material produced by a blade from a frit taken from a commercially available ~~Sep-Pak~~ SEP-PAK® C18 cartridge. The semi-permeable frit structure permitted the confinement of the irregularly shaped CPG beads containing immobilized Fab' fragments of IgG within the analyte concentrator-microreactor. The cavity was filled with CPG beads through an additional port (termed the filling port) after the appropriate tubing (two PEEK® plastic tubes and two fused-silica capillary tubes) at the four ports were previously installed. After the cavity was properly filled with the coated beads employing a low vacuum aspiration system and gentle shaking of the device, the filling port that facilitated the entrance of the beads into the system was closed very tightly to prevent air from entering the analyte concentrator-microreactor. Since the analyte concentrator-microreactor

device was made of a transparent acrylic substrate, the entire packing process was monitored using a stereo microscope.

[0171] The solid-phase microextraction device was designed with four microvalves as indicated by circles with cross areas in ~~Figure~~ FIG. 11A and in more detail in ~~Figures~~ FIGS. 25-27. The micro-fabricated valves permitted full control of the path of fluid in the appropriate direction, allowing the interaction of the constituents of the sample under study with the antibody fragments present in the analyte concentrator-microreactor device.

[0172] Once the analyte concentrator-microreactor was completed packed and properly assembled, one of the inlet positions for the large-bore tubing was attached by a commercially available connector to a 3-mL plastic syringe as depicted in ~~Figures~~ FIGS. 30 and 31.

Please replace paragraph [0174] with the following paragraph:

[0174] Capillary electrophoresis studies for ibuprofen and naproxen [12], and angiotensin II and neurotensin [13,14] have been reported previously using commercially available instruments, but not using the cross-shaped analyte concentrator-microreactor configuration. For experiments directed to this application, a capillary electrophoresis apparatus as depicted in ~~Figure~~ FIG. 31 was employed [4,14]. The fused-silica capillary (100-μm x 65-cm x 100-cm) used for analyte separation was conditioned prior to being connected to the microextraction device, by rinsing with water for 2 min, 0.5 N NaOH for 5 min, water for 3 min, and with background electrolyte (as specified below) for 5 min. The introduction of sample and washing buffer was achieved by using the appropriate valves to direct the flow of liquid (~~Figures~~ FIGS. 11A and 25-27). When the valves for the transport tube were closed, the separation capillary was conditioned with 50 mM sodium tetraborate, pH 8.5. When

the valves for the separation capillary were closed, samples were introduced into the analyte concentrator-microreactor using a large-bore plastic tube by positive pressure using a syringe, or by employing a low vacuum aspiration system directly from the sample reservoir. The amount of sample introduced into the microextraction device was approximately 1 mL, a sufficient volume containing enough concentration of hapten to saturate the binding sites of the antibody fragments. Standards were prepared in 50 mM sodium tetraborate buffer, pH 9.0. Spiked urine samples were prepared by adding the analytes directly to undiluted urine, or to diluted urine in 50 mM sodium tetraborate buffer, pH 9.0. The sample was allowed to be in direct contact with the immobilized affinity ligand for 5 min, permitting the peptide to be retained in order to control the appropriate interaction and temperature for achieving maximum binding. After a few washes of the transport tube with 50 mM sodium tetraborate buffer, pH 9.0, the valves were switched to the separation position. The separation column was conditioned once more with 50 mM sodium tetraborate buffer pH 8.5, and completely degassed. The peptide was finally eluted with a plug of approximately 100 nL of 300 mM glycine-HCl buffer, pH 3.4, or preferentially 100 nL of 10 mM phosphate-buffered saline, pH 7.4, containing 20 - 50% (v/v) acetonitrile, and the separation was allowed to continue. (The glycine buffer is used normally when the peptides are labeled with a fluorescence chromophore, and fluorescence detection is employed. The phosphate-buffered saline, containing acetonitrile or other organic solvent, is normally used when the peptides are not tagged by a fluorescence chromophore, and ultraviolet detection is employed.)

Please replace paragraph [0178] with the following paragraph:

[0178] Improved procedure to obtain Fab' fragments. (~~Figure~~ FIG. 20.)

Please replace paragraph [0180] with the following paragraph:

[0180] In the experiments reported here, it was confirmed that the removal of N-linked carbohydrates was preferred for facilitating pepsin activity on the IgG molecule, to generate dimeric F(ab')2 fragments and then monomeric Fab' fragments (see ~~Figure~~ FIG. 20). Comparative pepsinolysis studies of IgG, with and without glycosylation, demonstrated a more consistent yield of the monomeric deglycosylated IgG when qualitative and quantitative capillary electrophoresis studies were performed (data not shown). Covalent attachment of monomeric Fab' fragments through SH- groups permits proper orientation of the molecule and increases the surface area to enhance the capturing of a target ligand. Specifically oriented attachments of antibodies, or antibody fragments, to a surface have been demonstrated to be more efficient in capturing a target analyte, when compared to chemistries employing random attachments [22-24]. The advantages of oriented immobilization of biologically active proteins are: (a) improved steric accessibility to the active binding sites; (b) increased stability of the immobilized molecule; and (c) facilitation to a greater surface area of affinity interaction.

Please replace paragraph [0182] with the following paragraph:

[0182] Determination of non-steroidal anti-inflammatory drugs and neuropeptides in urine was carried out by immunoaffinity capillary electrophoresis. Several experiments were performed to test the efficiency of the system. In a preliminary study, a 50-ng/mL solution of angiotensin II was applied to the analyte concentrator-microreactor device containing immobilized Fab' fragments derived from a polyclonal antibody raised against the peptide. The device was part of the capillary electrophoresis instrument depicted in ~~Figure~~ FIG. 31. The sample was allowed to be in direct contact with the immobilized affinity ligand, the peptide was retained, and after a few washes and conditioning with the appropriate buffers, the peptide was eluted with a small plug of 0.3 M

glycine-HCl buffer, pH 3.4, or neutral pH buffers containing acetonitrile, such as phosphate-buffered saline, pH 7.4, containing 20 - 50% acetonitrile, or other organic solvents at concentrations ranging from 5% to 100% (apparently, there is not a universal way of eluting all antibodies or antibody fragments. It is preferred to optimize every elution condition for each individual immobilized affinity ligand-target analyte complex. Since the affinity binding is different for every immunological complex, it may affect the linearity of quantification if the hapten is not released completely from the immunological complex). The success of this experiment prompted optimization of the binding conditions. A series of dilutions were performed in the urine specimen, and spiked with the acidic drugs or peptides. A representative electropherogram is shown in ~~Figures~~ FIGS. 32A and 32B. The urine specimen used in this experiment was diluted 1:1 (v/v) with 50 mM sodium tetraborate buffer, pH 9.0. The sample was divided in two aliquots. One aliquot was spiked with 5 ng/mL each of ibuprofen and naproxen, and another with 5 ng/mL each of angiotensin II and neurotensin. The samples were analyzed in two separate analyte concentrator-microreactors. The data obtained was consistent for 9 runs, maintaining reproducible migration times and peak areas as indicated in Table 1 below.

Please replace paragraphs [0186] through [0189] with the following paragraphs:

[0186] ~~Figures~~ FIGS. 33A, 33B, and 33C illustrate electropherogram for analytes detected using the electrophoresis apparatus 10 with three concentrators with a different antibody in each of the concentrators. In this case, three antibodies of Fab' fragments which act against the following peptide hormones were used: (A) neurotensin; (B) enkephalin; and (C) cholecytokinin. The concentrators 34, 36, and 38, each had an antibody with affinity towards (A) neurotensin; (B) enkephalin; and (C) cholecytokinin, respectively. Urine specimen were spiked with the

neurotensin, enkephalin, and cholecytokinin analytes prior to IACE. The urine specimen were then pass through the transport capillary 24 towards the three concentrators 34, 36, and 38. After the three antibodies captured their respective analytes, the separation capillaries 28, 30, and 32 were eluted sequentially. ~~Figure~~ FIG. 33 indicates that migration time and peak for the three analytes: (A) neurotensin; (B) enkephalin; and (C) cholecytokinin correspond the control sample illustrating that the electrophoresis apparatus 10 operates consistently for the analytes under study. The process of capturing each peptide from the urine specimen by immobilized antibody fragments located at the analyte concentrator, cleaning the capillary with an appropriate buffer, eluting the bound peptide from the immobilized affinity ligand, and separating the released peptide by capillary electrophoresis was carried under the same experimental conditions as described above in the Experimental Data.

[0187] ~~Figure~~ FIG. 34 illustrates a diagnostic kit 260 that may be used by individuals to detect early signs of certain disease(s). Some individuals may be predisposed to certain diseases more so than others based on their family health history, such as cancer, diabetes, and heart diseases. For these individuals, an early detection of such diseases may be a key to fighting the diseases. In this regard, individuals may use the diagnostic kit 260 to monitor and detect early signs of a number of diseases. Such tests may be done at the home of the individual for convenience and privacy. The diagnostic kit 260 may include the electrophoresis apparatus 10 that is ~~communicateably~~ communicatably coupled to a CPU 262 that may operate the electrophoresis apparatus 10 based on a predetermined set of instructions. As discussed above, the valves on the transport and separation capillaries may be motor operated, which are controlled by the CPU.

[0188] An individual that is predisposed to a predetermine disease may select or purchase a system of capillaries and valves with the concentrators 34, 36, and 38 that may isolate biomarkers that are associated with a predetermined disease. In general, each disease may have a plurality of biomarkers or analytes associated with that disease. A different disease may have different biomarkers than other diseases. As such, biomarkers may serve as a fingerprint for identifying a particular disease an individual may have based on test performed on the individual's specimen. If the biomarkers are detected, then evaluation may be made as to whether the biomarkers correspond to a particular disease or not. For instance, Disease 1 may be associated with four biomarkers: A, K, M, and T; Disease 2 may be associated with five biomarkers: B, D, F, L, and P; and Disease 3 may be associated with three biomarkers: B, T, and Y. Each biomarker may have its migration time through the separation capillary and peak that may be detected by the detector 86. If an individual is predisposed or concern about the disease 2, then the individual may select a system of capillaries and valves with at least five analyte concentrators where each analyte concentrator has an affinity towards the analytes or biomarkers B, D, F, L, and P, respectively, or in any order. In the case of detecting disease 3 with three biomarkers, concentrators 34, 36, and 38 as illustrated in ~~Figure~~ FIG. 9 or ~~Figure~~ FIG. 14 may be used to isolate biomarkers B, T, and Y, respectively. Alternatively, as illustrated in ~~Figure~~ FIG. 22, a separation capillary 28 having three types of antibodies 140, 150, and 160 within its interior wall between the valves 104 and 108 may be used to isolate the biomarkers B, T, and Y, in any order. Likewise, the separation capillaries 30 and 32 may be used to isolate biomarkers A, K, M, and T for the disease 1, and biomarkers B, D, F, L, and P for the disease 2, respectively. As such, one system of capillaries and valves may be used to isolate biomarkers for more than one disease.

[0189] The individual may install the system of capillaries and valves into the platform 12 and locked it in placed with the holders 49. For isolating the biomarkers, the individual's specimen such as urine may be provided into the sample cup 15. Other ~~specimen~~ specimens such as blood, hair, and nail may be provided. The CPU may then send the control signals 266 to operate the apparatus 10 according to the steps generally discussed in ~~Figure~~ FIG. 12 to isolate the analytes of interest or the biomarkers from the specimen provided by the individual. The detector 86 may then obtain the data for each of the biomarkers in terms of their respective migration time and peak. For instance, if the individual providing the specimen does have the disease 3, then the detector 86 may find three biomarkers B, T, and Y, each having its respective migration time through the separation capillary and peak. On the other hand, if the individual does not have the disease 3, then one or two of the biomarkers may be detected from the specimen but not all three biomarkers. This data information 268 may be analyzed in a variety of ways. For instance, the data information 268 may be provided to the CPU 262, which is then compared with the plurality of reference data stored in the memory 264. The CPU may find that the biomarkers do indicate that the specimen provided by the individual has the disease 2 if all three biomarkers are found to have substantially similar respective migration time and peak as compared to the migration time and peak indicated in the reference data stored in the memory 264. On the other hand, if at least one of the biomarkers do not substantially match up with the migration time and the peak, then the CPU may indicate that the individual may not have the disease 2.